National Aeronautics and Space Administration

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Lyophilization as a Potential Means of Preserving Microorganisms for Space Environment Radiobiological Studies

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INTRODUCTION

NASA has set goals for manned-spaceflight missions beyond low-Earth orbit. It has been suggested that the most critical issue that needs to be addressed in order to safely reach these destinations (i.e. the moon, Mars, and asteroids) is the hazard of space radiation.[1] Current estimations of the oncogenic risks involved with certain amounts of exposure are subject to many uncertainties due to the limited data available.[2] Microorganisms are excellent test subjects for radiobiological research in orbiting spacecraft (as well as robotic planetary missions) due to their low weight/cost and wide range of metabolic activity. They will also likely play a vital role in long-term manned missions serving as a means of carbon/nitrogen cycling and oxygen production. A better understanding of the effects of, and how to protect organisms from, solar and cosmic radiation is imperative if these longer-duration spaceflights are to be attempted. This work investigates lyophilization as a means of maintaining microorganisms in a period of stasis without the need to store them in extremely cold temperatures.

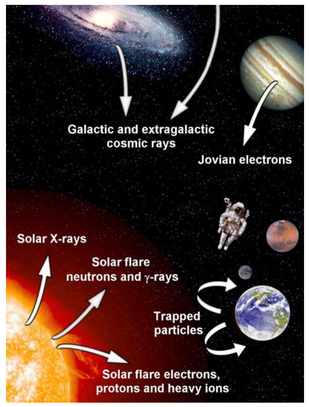
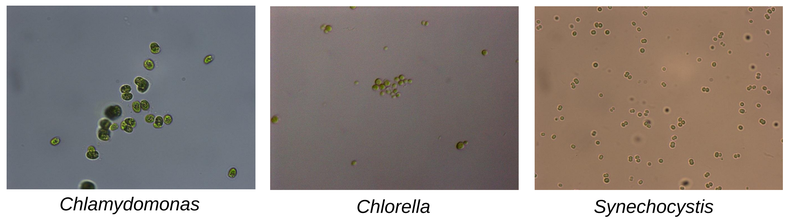


Figure 1. Radiation Sources in Space
(From Hellweg and Baumstark-Khan, 2007)

MATERIALS AND METHODS

The primary organisms utilized in this study were two algae, *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, and a cyanobacterium, *Synechocystis sp. PCC 6803*.



Chlamydomonas *Chlorella* *Synechocystis*

Figure 2. Microorganisms Tested. *Images were taken with a Nikon Microphot-FXA microscope at 400x magnification.*

- *Chlamydomonas*, *Chlorella*, and *Synechocystis* were grown in 50 mL of P49, 847, and BG-11^{+} media respectively, in 125 mL flasks on a rotary shaker (70 rpm) under constant lighting (≈ 60 µmol photons·m^{-2}·s^{-1}), and standard growth curves were generated over a period of 25 days by monitoring optical densities at a wavelength of 750 nm.
- Cell counts for *Chlamydomonas* and *Chlorella* were carried out using a hemocytometer and correlated to optical density. (*Synechocystis* proved to be too small to count with this method.)
- Preliminary tests of 3 cryopreservatives were attempted: 50% glycerol, 10% dimethyl sulfoxide (DMSO), and 3% methanol (in the respective medium for each organism). These were monitored on only a growth or no growth basis after organisms were kept in a -20˚ C freezer (for glycerol and DMSO) or a -80˚ C freezer in an isopropanol bath (for methanol) overnight and then placed in media.
- Prior to lyophilization, 1 mL aliquots of sample were pipetted into 1.5 mL centrifuge tubes and placed in a -20˚ C freezer for 30 minutes. These frozen samples were left opened, the tops covered with aluminum foil, and then were lyophilized.
- After the first lyophilization trial, samples were placed in the dark for 3 days, and then in a 12 hour light/dark cycle. After the second lyophilization, samples were immediately placed under constant lighting.

RESULTS

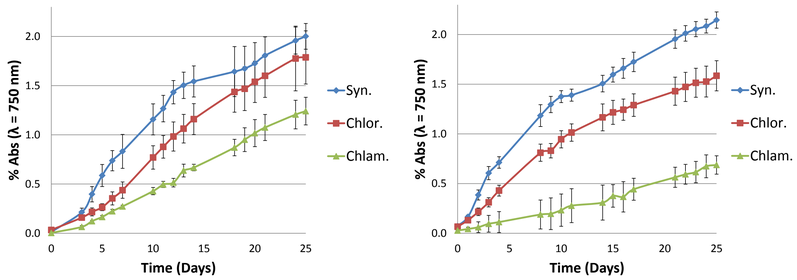


Figure 3. Standard Growth Curves. *Two separate growth curves for each organism were generated over 25 days. For each run the three organisms were grown in triplicates. The data shown above are the averages and standard deviations of the triplicates for each individual organism.*

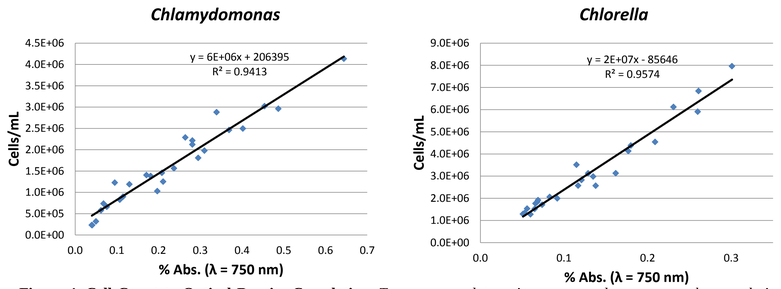


Figure 4. Cell Count to Optical Density Correlation. *Twenty-seven data points were used to generate the correlation chart for Chlamydomonas and twenty-five were used for Chlorella.*

Table 1. Preliminary Cryopreservative Tests.*

	50% Glycerol	10% DMSO	3% Methanol
Synechocystis	-	+	Not Tested
Chlorella	-	+	Not Tested
Chlamydomonas	-	-	+

**A '+' indicates the organism survived freezing with the corresponding cryopreservative.*

In both trials, *Synechocystis* survived the lyophilization process.

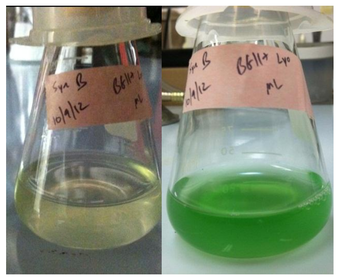


Figure 5. 1st Run Post-Lyophilization *Synechocystis*. *Left is day 0 in medium. Right is day 29.*

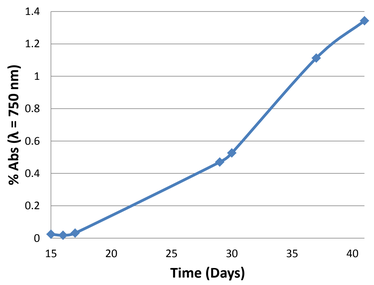


Figure 6. 1st Run Post-Lyophilization *Synechocystis*. *Optical densities were not recorded until Day 15.*

DISCUSSION/SUMMARY

- Freezing tests with no cryopreservatives showed some viability in *Synechocystis* and *Chlorella*, however *Chlamydomonas* did not survive.
- It was found that 10% DMSO as a cryopreservative was effective in increasing survivability for *Synechocystis* and *Chlorella* after freezing, while 3% methanol was successful in retaining some viable cells for *Chlamydomonas*.
- *Synechocystis* shows promise as it was the only organism to survive lyophilization, and did so even without any cryopreservative.

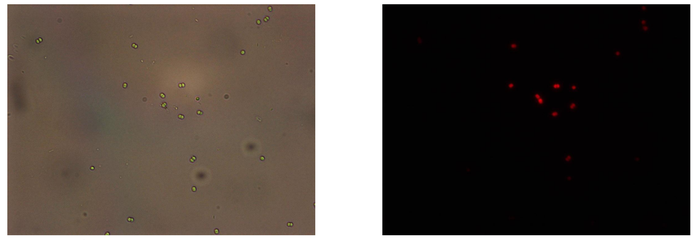


Figure 7. 2nd Run Post-Lyophilization *Synechocystis* Fluorescence. *Chlorophyll fluorescence was detected when first examined 6 days after lyophilization.*

CONCLUSION

The only microorganism that has survived the lyophilization procedure is the bacterium Synechocystis - even without any cryopreservative. It is presumed to be worthwhile to pursue the use of cryopreservatives, or lyopreservatives such as sucrose (if heterotrophic contamination can be controlled), in conjunction with the lyophilization process to try and improve the amount of cells recoverable. It has been suggested that growing organisms in a cold environment for weeks before freezing them can substantially increase their tolerance to being frozen.[3] Future plans also include to investigate microorganisms already known to be tolerant to desiccation. For instance, *Nostoc comune* has been shown to still contain viable cells after having been dried out for over 100 years, and cyanobacteria of the genus *Chroococcidiopsis* are known for their survival abilities in harsh desert environments.[4,5] It is also important to mention that the goals of this study were to examine only the effects of cryopreservation and lyophilization on viability over very short time scales. The samples were only kept in their desiccated state for a matter of days, so plastic centrifuge tubes were acceptable. For longer storage times, glass is recommended as water molecules can pass through plastic, prematurely rehydrate the cells, and decrease the sample's viability overtime.

ACKNOWLEDGMENTS

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REFERENCES

[1]Hellweg, C. E., and Baumstark-Khan, C., "Getting Ready for the Manned Mission to Mars: The Astronauts' Risk from Space Radiation," *Naturwissenschaften*, 2007, 94:517–526.
[2]Cucinotta, F. A., et al., "Space Radiation Cancer Risks and Uncertainties for Mars Missions," *Radiation Research*, Vol. 156, No. 5, November 2001, pp. 682-688.
[3]Ben-Amotz. A., and Gilboa. A., "Cryopreservation of Marine Unicellular Algae," *Marine Ecology – Progress Series*, Vol. 2, 30 April 1980, pp. 221-224.
[4]Cameron, R. E., "Species of the Nostoc Vauch. Occuring in the Sonoran Desert in Arizona," *Trans Am Microsc Soc*, Vol. 81, 1962, pp. 379-384.
[5]Grilli Caiola, M., Ocampo-Friedmann, R., and Friedmann, E. I., "Cytology of Long-term Desiccation in the Desert Cyanobacterium Chroococcidiopsis (Chroococcales)," *Phycologia*, Vol. 32, No. 5, September 1993, pp. 315-322.